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REVIEW ARTICLE

COX-2, TNF-α AND APOPTOSIS : NEWER STRATEGIES IN INFLAMMATORY DISORDERS

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ABSTRACT

Inflammatory conditions related to rheumatoid arthritis, injury and infection necessitates the need for the use of nonsteroidal antiinflammatory drugs (NSAIDs) to curb these ailments. There have been high levels of concern regarding their use since extensive and indiscriminate use of NSAIDs results in toxicity. The need for an overall therapeutic benefit with no or reduced toxicity has envisaged the need for investigating newer sites of antiinflammatory activity. This article highlights the development of selective COX-2 inhibitors, and newer concepts of antiTNF-α therapy and induction of apoptosis as potential strategies in combating inflammation.

Nonsteroidal antiinflammatory drugs (NSAIDs) form a class of therapeutic substances that are most widely used world over because of their analgesic, antipyretic and antithrombogenic effect besides antiinflammatory activity. It is estimated that more than 30 million people world wide take NSAIDs daily[1], and that 40% of these individuals are older than 60 years of age[2]. The estimated annual market of these agents is about US $ 6 billion[3]. NSAIDs have found an indispensible place in the treatment of rheumatic disorders. About 1 to 2% of population in many regions have rheumatoid arthritis (RA), while osteoarthritis (OA) affects about 10% of the world's population, of which 50% are the elderly population. As a result, the past few years have seen a significant rise in the prescription of NSAIDs. Further, besides being used in the treatment of RA and OA, NSAIDs have been increasingly used in the treatment of acute and non-inflammatory conditions, including acute musculoskeletal disorders, low back pain, acute gout, symptoms of common cold and primary dysmenorrhea.

Table 1 give the list of antiinflammatory agents currently used. It is noted that all of them have the same mechanism of action like that of aspirin, hence they are also known as "aspirin-like drugs".

Mechanism of action:

The mechanism of action of these drugs, particularly that of aspirin has been the subject of great interest. Vane and his colleagues in 1971 demonstrated that aspirin and related drugs inhibit prostaglandin (P) biosynthesis[4]. NSAIDs inhibit the enzyme prostaglandin endoperoxide synthase or cyclooxygenase (COX) which is involved in

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Table 1. Classification of NSAIDs

- Salicylic acid derivatives
  (e.g., Aspirin, diflunisal)
- Para-aminophenol derivatives
  (e.g., Acetaminophen)
- Indole and indene acetic acid
  (e.g., Indomethacin, sulindac, etodolac)
- Heteroaryl acetic acids
  (e.g., Tolmetin, diclofenac, ketorolac)
- Aryl propionic acids
  (e.g., Ibuprofen, naproxen, flurbiprofen)
- anthranilic acids (fenamates)
  (e.g., Mefenamic acid, meclofenamic acid)
- Enolic acids
  (e.g., Oxicams - piroxicam,
  Pyrazolidinediones - phenylbutazone)
- Alkanones
  (e.g., Nabumetone)

prostaglandin synthesis. Only the cyclooxygenase function of the enzyme is inhibited[5], and the inhibition is brought about by the steric blockade of the receptor active site channel. This may be due to either a chemical transformation of the enzyme residue as in case of irreversible acetylation by aspirin[6], or a conformational modification of the enzyme as seen in inhibition with other NSAIDs like indomethacin[7], etc.

Side effects:

While NSAIDs are effective in the management of pain and inflammation in a large number of conditions, it is now well established that they cause upper gastrointestinal (GI) damage including lesions, 'silent' ulcers, and life threatening perforations and haemorrhage. In addition to upper GI damage, NSAID therapy has been found to be associated with damage to the lower GI tract (i.e. small and large intestine)[8]. Substantial mortality has been found to be associated with the long term use of NSAIDs in the management of rheumatic diseases. NSAID use represent a threat to life in patients with rheumatic disease that is second only to RA and its complications. Infact, approximately 7600 RA patients in the United States die each year from GI complications associated with NSAIDs use[9].

Interference with both the control of vascular resistance and the regulation of extracellular volume homeostasis has been incriminated in NSAID induced hypertension[10], but several other putative mechanisms such as moderation of adrenergic activity or resetting of the baroreceptor response may also be involved. The increment in blood pressure seems to be larger in hypertensive than in normotensive patients. Nevertheless, it seems reasonable to assume that the effects of NSAIDs on blood pressure will be greater when the initial blood pressure is higher.

Renal blood flow and glomerular filtration rate become progressively dependent upon PG synthesis under conditions of volume depletion or reduced renal perfusion pressure. Classical clinical examples of the later are congestive heart failure, renovascular hypertension and cirrhosis of the liver, activation of renin-angiotensin system being the common denominator. Administration of NSAID to such patients may cause acute renal failure[10].

Long-term NSAIDs may cause infertility in women. Ultrasound scans reveal that the follicles produced fail to rupture to release eggs. The process of follicular rupture is PG dependent and could be blocked by NSAIDs. Human semen and other regions of the male reproductive system are abundant sources of PG. The role of PG in the male fertility is not well elucidated and it can be speculated that

prolonged use of NSAIDs could lead to infertility and impotence in males. However, no reliable data on this account is available.

Because of the substantial risks involved with the long term use of NSAIDs there is an increasing demand for the development of newer agents with better pharmacological profile. The major impetus for the development of new NSAIDs is SAFETY, i.e. to improve NSAID tolerability, particularly gastric tolerability.

COX-2 Isoenzyme & Selective COX-2 Inhibitors

The 1990s has seen a new dawn in inflammation research, with several studies demonstrating increased COX activity in a veriety of cells after exposure to endotoxins[11], inflammatory cytokines[12], growth factors[13], hormone[14], and tumor promoters[15]. This gave rise to the new concept that there might be a constitutive COX activity, further referred to as COX-1 and an 'inducible one,'further referred to as COX-2[16]. The discovery of COX isoenzymes has given rise to a better understanding of the inhibition of COX by classical NSAIDs and offer the prospect of devising new and potentially safer drugs.

The inducible cyclooxygenase (COX-2) shares 60% aminoacid identity with COX-1[17,18]. It also has a similar mechanism of arachidonic acid metabolism. They are large soluble globular proteins with no transmembrane region. The two isoenzymes have about the same affinity (Km) and capacity (Vmax) to convert arachidonic acid to prostaglandin endoperoxide (PGH_2)[19]. However, they are pharmacologically distinct and show differential sensitivity to inhibition by NSAIDs (Table 2)[15,20-32].

The results of recent studies show that COX-2 enzyme is dimeric, each monomer consisting of a catalytic domain (containing haem) and a membrane-binding domain, connected by the N-teminal EFG domain. The membrane-binding domain forms a channel which leads to the active site and the binding site for NSAIDs. The presence of a side pocket in the binding channel accounts to extended binding of inhibitors side ways with in the channel, there by enhancing specificity for COX-2. Slow dissociation from this side pocket may explain the time dependency of inhibition of COX-2 by some NSAIDs[33].

COX-2 RNA can be induced experimentally in the hippocampus of rats as a result of seizures. Concomitant release of platelet-activating factor (PAF) by the neuron also cause an increase in COX-2 message. This suggest that PAF induction of COX-2 is an integral component of neuronal death during seizures[33].

Human microglia, the phagocytic cells of the CNS, express COX-2 under pathological conditions, and the inhibition of the enzyme in these cells and the reduction of neuronal apoptosis may underlie the recent discovery that clinical administration of NSAIDs can delay the symptoms of Alzheimer's disease[33].

COX-2 has also been attributed a role in vascular diseases. Platelet micro-particles contain high concentration of arachidonic acid and can induce COX-2 and PGI_2. They inhibit platelet aggregation and may be responsible for the automodulatory action of platelets on their own function.

COX-2 is relatively insensitive to inhibition by low doses of aspirin. Occasionally, aspirin-resistant thromboxane synthesis can arise episodically in patients with unstable angina due to activity of COX-2 in monocytes infiltrating the damaged vascular tissue, which could provide PGH_2 for transcellular production of pro-aggregatory thromboxane A_2 by platelets.

The discovery of inducible PG synthase, distinct from the constitutive enzyme, renewed interest in

Table 2 Differences between COX-1 and COX-2 isoenzymes

COX - 1	COX – 2
Physiology:	
• Constitutive form of COX	• inducible form of COX
• "house keeping gene" to produce PG that help regulate normal kidney and stomach function and vascular homeostasis	• "Inflammatory response gene" induced during inflammation, produce PG involved in inflammation "immediate early gene" thought to control mitogenesis; may produce PG involved in cell growth
Localization	
• present in platelets, endothelial cells, stomach, kidney, smooth muscle, most tissues	• present in brain - cortical & limbic neurons, activated monocytes or fibroblasts and synoviocytes during inflammation and in follicles preceeding ovulation.
• lumen of ER	• ER and nuclear envelop
Amino Acids :	
• 599 amino acids	• 604 amino acids
• a cassette of 17 a.a. sequence near the N-terminal that is absent in COX-2	• a cassette of 18 a.a. sequence near the C-terminal that is absent in COX-1
• N-terminal sequence begins with ADPGA	• N-terminal sequence begins with ANPCC
Molecular Weight :	
• 72000	• 74000
Regulation of Expression :	
• gene is 22 Kb, with 11 exons	• gene is 8.3 Kb, with 10 exons
• gene located on chromosome 9	• gene located chromosome 1
• mRNA transcript is 2.8 to 3.0 Kb	• mRNA transcript is 4.0 to 4.5 Kb
• mRNA transcript is not degraded fast	• mRNA transcript is degraded quickly
• promoter region of gene has poor inducibility	• promoter region contains many transcriptional factors which can be upregulated by pro-inflammatory cytokines
• post transcriptional addition of 3 high mannose oligosaccharides	• post transcriptional addition of 4 high mannose oligosaccharides
• not inhibited by glucocorticoids	• inhibited by glucocorticoids
Active site :	
• smaller active site	• larger active site
may be because Isoleucine in COX-1 is replaced by smaller valine in COX-2	
Substrate	
• Only C_{20} carboxylic acids	• Both C_{18} and C_{20} carboxylic acids
Phase of inflammation:	
• Main source of PG in chronic inflammation phase	• Main source of PG in acute inflammation phase
Acetylation by Aspirin :	
• Acetylation of Ser 530	• acetylation of Ser 516
• complete inhibition of COX activity	• modification of enzyme to produce 15 hydroxyeicosatetraenoic acid (15-HETE)

developing new non-steroidal antiinflammatory drugs for the therapy of inflammation. The identification of COX-2 isoenzyme led to the hypothesis that it might be responsible for the production of protaglandins at inflammatory sites. Thus, the selective inhibition of this isoenzyme would reduce inflammation without the side effects of gastric and renal toxicity[34].

Classical NSAIDs like aspirin inhibit both COX-1 and COX-2. It is believed that selective inhibition of COX-2 is desirable for antiinflammatory action. If the new COX-2 selective NSAIDs can effectively inhibit inflammatory PG synthesis by COX-2 without inhibiting COX-1 PG synthesis, required to regulate sodium and water reabsorption and renal blood flow, it is likely that these new drugs will also have significantly less renal toxicity than present day NSAIDs.

NSAIDs can be ranked by the concentration of drug necessary to achieve 50% inhibition (IC_{50}) for COX-2 divided by the IC_{50} for COX-1 (both in µg/ml), and value below unity indicates selectivity for COX-2.

$$\frac{IC_{50}\ COX\text{-}2}{IC_{50}\ COX\text{-}1} < 1 \text{ implies COX-2 selectivity}$$

Table 3 shows few classical and selective COX-2 inhibitors, the assay system used and their selectivity indices.

Selectivity COX-2 inhibitors exhibit 100 to 1000 fold selectivity for the inducible form of COX. Animal studies show hardly any intestinal damage after very large dose of highly selective COX-2 inhibitors[34]. The development of highly selective COX-2 inhibitors may, therefore, represent a major advance in the quest for a safe NSAID. In humans, several compounds, such as L-745337, T-614 and celecoxib, are in preclinical development or early clinic trials, while others such as NS-398 and flosulide, have been discontinued because of reported nephrotoxicity on post marketing surveillance. Nimesulide and meloxicam have been studied in well controlled clinical trials and have been found to be atleast as effective as the classical NSAIDs, both in painful diseases and chronic inflammatory joint disease.

A significant increase in COX-2 occur in amnion from patients delivering following labour compared to those delivering by elective caesarean section. It is thus likely that COX-2 produces the oxytocic PG that are responsible for labour. This suggests that increased COX-2 expression may inititate preterm labour associated with infection. These data indicate that selective COX-2 inhibitor may be of use in preventing contractions in premature labour, being preferable to β-sympathomimetics (which produces maternal cardiovascular, respiratory and metabolic side effects) and indomethacin (which produces oligohydramnios and closure of the ducts arteriosus due to reduced synthesis of vasodilator PGs).

Several animal and epidemiological studies show that NSAIDs have an antineoplastic action[37]. Normal colon tissues express COX-1 where as the colonic tumors express high levels of COX-2[38]. Thus the development of more specific inhibitors of COX-2 may provide useful cancer chemopreventive agents. The actual involvement of COX-2 in the neoplastic transformation is suggested by the causation of programmed cell death (apoptosis) after administration of NSAIDs. The ability of these drugs to induce apoptosis closely parallel their ability to inhibit COX-2. Thus, the main appeal of these new NSAIDs (selective COX-2 inhibitors) lie in an improved safety profile and the wider therapeutic use they can be put into.

TUMOR NECROSIS FACTOR - α (TNF - α)

TNF-α is an early response cytokine which is

Table 3:Selectivity indices of some NSAIDs

Abbreviations : IC_{50} = Concentration at which 50% of activity is inhibited; h=human; rh = recombinant human; IL-1 = cells treated with IL-1

Drug	ASSAY FOR		IC_{50}(μM)		RATIOS
	COX-1	COX-2	COX-2	COX-1	COX-2/COX-1
Naproxen	hCOX-1	hCOX-2	15.00	0.20	75.00
Indomethacin	hCOX-1	hCOX-2	1.00	0.02	50.00
Aspirin	Purified COX-1	Purified COX-2	1166.00	27.75	42.00
Ibuprofen	hCOX-1	hCOX-2	20.00	5.00	4.00
Ketorolac	hCOX-1	hCOX-2	61.50	31.50	1.95
6-MNA	hCOX-1	hCOX-2	110.00	70.00	1.57
Nimesulide	rhCOX-1 Sf9 (purified)	rh COX-2 Sf9 (purified)	1.30	70.00	0.0186
Meloxicam	rhCOX-1 Sf9 (purified)	rhCOX-2 Sf9 (purified)	0.50	37.00	0.0135
SC - 58125	Platelets	IL - 1 - fibroblast	0.07	100.00	0.0007
CGP - 28238	Human platelets	IL-1-rat mesangial cells	0.0147	72.30	0.0002

(Ref. 35,36)

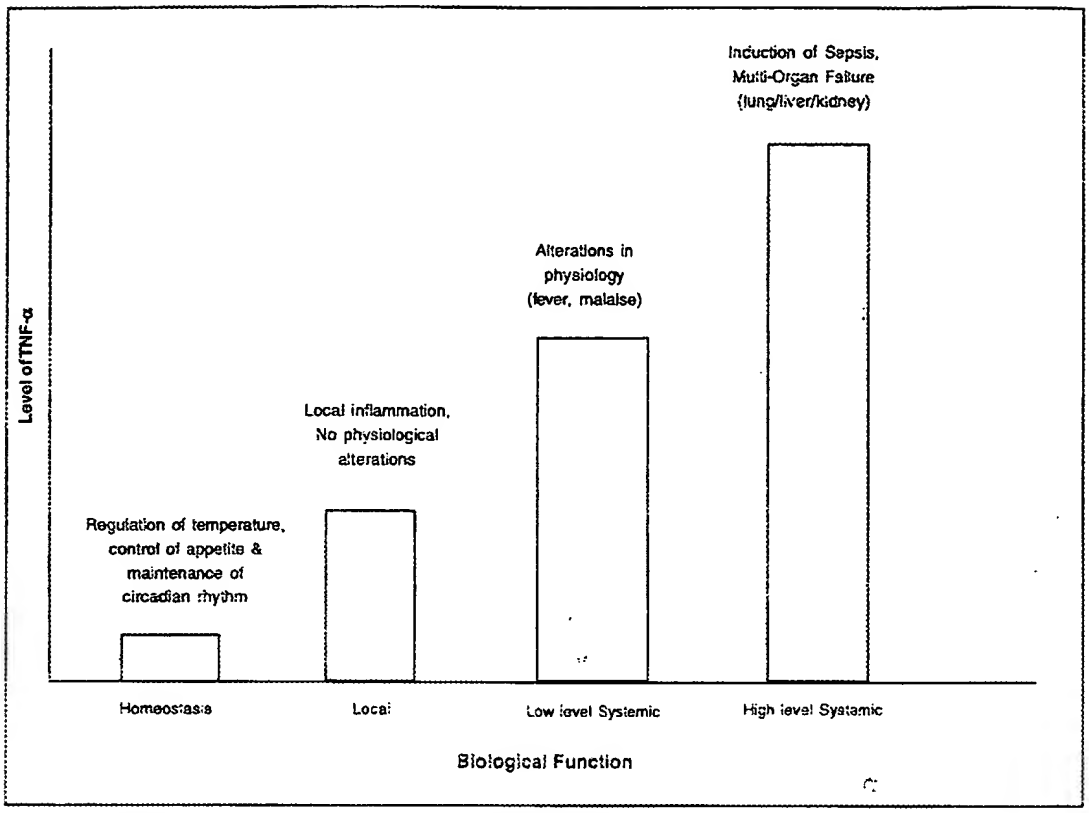
involved in the pathogenesis of various inflammatory conditions besides its physiological role in normal host defense mechanism. It is primarily produced by monocytes and lymphocytes.

In 1891, William B. Coley reported haemorrhagic necrosis of tumor following administration of bacterial tissue to tumor bearing animals. However, what exactly brought about the haemorrhagic necrosis could not be accounted for. In early 1960's, Dr. Valy Menkin described the presence of a factor liberated by injured cells which when injected into the cutaneous tissue of rabbit caused redness, necrosis and swelling. He called this injurious factor 'necrosin'. In 1975, Carswell[39] described a factor which was liberated in response to injection of lipopolysaccharide into tumor bearing mice and caused haemorrhagic necrosis in them. Since it caused necrosis of tumor he called it 'tumor factor'. In 1980, Beutler and Cerami described a factor derived from activated macrophages which they termed 'cachectin', which was postulated to be responsible for the syndrome of cachexia and endotoxin shock[40]. Later, in 1985, TNF was isolated and its amino acid sequence deduced. Today it appears that TNF, necrosin and cachectin are identical.

TNF-α is a hormone like polypeptide whose biologic function depends upon its relative concentration as shown by Fig. 1.

TNF-α plays important role in the normal host defense function and other vital activities as outlined in Fig. 2. Nevertheless, TNF-α is also implicated in many inflammatory conditions (Table 4)[41-49]. Its role in inflammation is depicted in Fig. 3. Excessive production of TNF-α to the level where they become systemically available in the blood stream can lead to inflammatory involvement with multiple organs, including lungs. In the worse case scenario, multi-organ failure may occur. The involvement of TNF-α has been extensively studied using animal models of the diseases[50,51] and an array of anti TNF-α treatments developed have proved to be efficacious in them.

Fig. 1: Change in biological function with increasing level of TNF-α



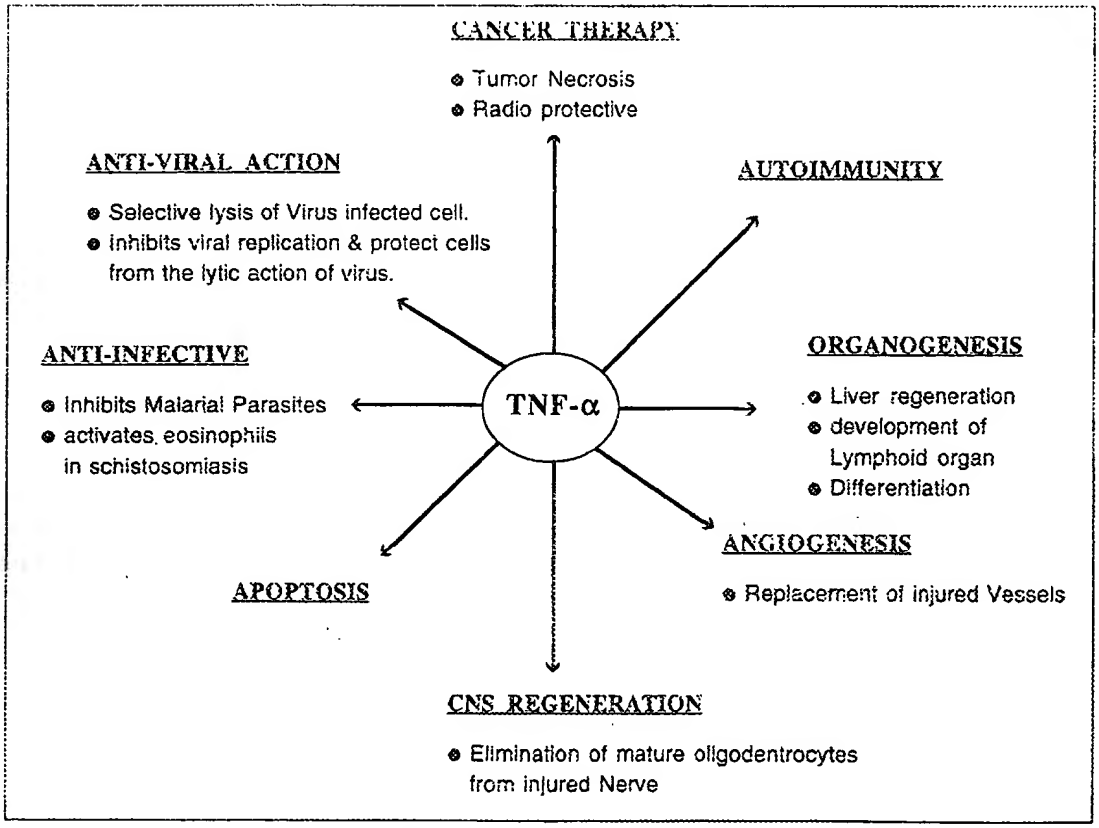


Fig. 2: Beneficial role of TNF-α

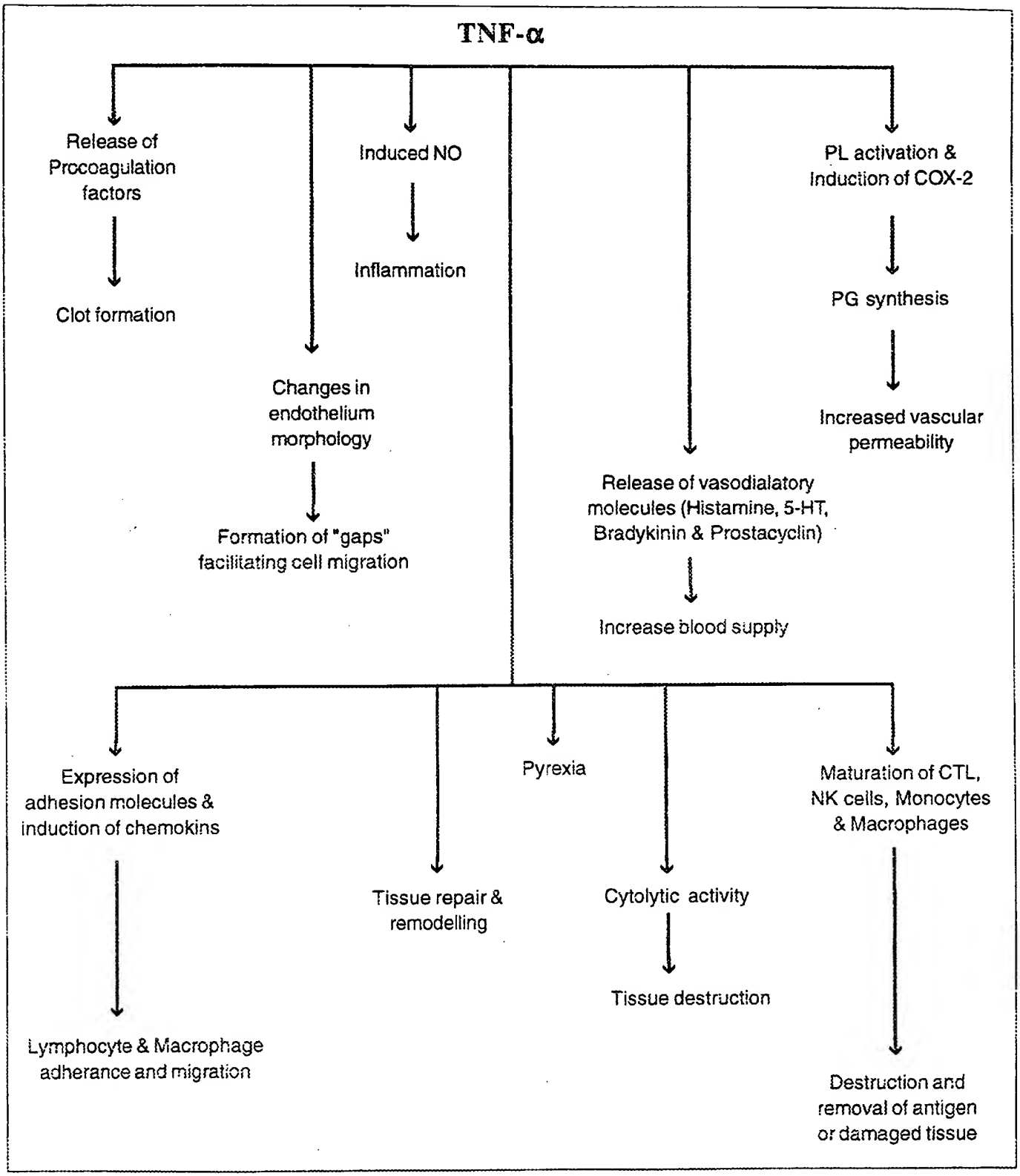


Fig. 3: Effects mediated by TNF-α during inflammation

Table 4. TNF-α in the pathogenesis of diseases

Human disease	Animal models of the disease
Cachexia	hTNF-α transgenic mice, tumor bearing nude mice
Septic shock syndrome	LPS alone, LPS/galactosamine and P. acnes / LPS - induced shock
Hepatitis	LPS, acetaminophen-induced hepatitis
Dermatitis	LPS-induced dermatitis, UVB or UVC radiation
Multiple sclerosis	Experimental allergic encephalomyelitis
Inflammatory bowel disease	DSS, TNBS-induced colitis
Pulmonary fibrosis	PMA, fiberglass, bleomycin-induced arthritis
Systemic lupus erythematosus	MRL - lpr / lpr mice, anti -DNA MAb in BALB/c mice
Rheumatoid arthritis	hTNF-α transgenic mice, adjuant-induced arthritis
Cerebral malaria	Murine model of cerebral malaria
CNS stress	Closed head injury in rats
Asthma	Allergic airway inflammation in guinea pig
Sarcoidosis	Chronic granulomatous inflammation of lungs in SCID mice
Meningiococcal meningitis	*Listeria* - mediated meningitis in mice
Alzheimer's disease	hTNF-α transgenic mice

Anti TNF-α Therapy

The various approaches against TNF-α include:

1. TNF-α converting enzyme (TACE) inhibitors

TACE has been found to be a specific zinc containing metal proteinase that cleaves peptide chains between alanine and valine residue. Metalloproteinase inhibitors nonspecifically inhibit the release of TNF-α, inhibit the shedding of TNF-α receptor protein P^{55} and P^{75}, and receptors for IL-6, IL-1 and IL-2 from cell surfaces. Examples of TACE inhibitors include matrix metalloproteinase (MMP) inhibitors like Marimastat, Batimastat, TAPI (TNF-α processing inhibitor), BB-1101, BB-2284, BB-3241, BB-2275, BB-2116, RO-31-9790, CBS-27023 A, SB-20358.

2. Phosphodiesterase IV inhibitors

Enhancement of intracellular cyclic AMP (cAMP) level has long been associated with antiinflammatory and antiallergic activity. The recognition of the existence of multiple, distant cyclic nucleotide phosphodiesterase (PDE) isoenzymes has generated considerable interest in the possibility of selective inhibitors of inflammation. PDE IV is the predominant cAMP-specific phosphodiesterase in inflammatory cells[52,53]. PDE IV inhibitor increases intracellular cAMP concentration thereby inhibit the release of TNF-α and related infiltration of cells as well as the production of interleukins. Examples of PDE IV inhibitors include Rolipram, Piclamistat, Isobutylxanthine, RO-20-1724, SB-207499, SK&F-95654 and CDP-840.

3. Thalidomide

Thalidomide, once known for its marked teratogenicity, is being viewed at with renewed interest because of its ability to degrade TNF mRNA, thereby inhibiting TNF-α production. It has been successfully used in the treatment of rheumatoid arthritis[54] and systemic lupus erythematosus[55]. US-FDA has also approved thalidomide for an expanded access trial for treatment of cachexia in AIDS patients.

4. TNF-α Inhibitors

Studies have shown the severity of disease in experimental allergic encephalomyelitis (EAE) to be reduced by treatment with TNF-α inhibitor pentoxifylline[56]. A similar result was also seen in the animal model for systemic lupus erythematosus with penotoxifylline[57].

5. Monoclonal antibody

Antibodies to TNF-α have been found to reduce disease activity in Crohn's disease[58]. Antibodies to TNF-α have also shown to prevent death in animal models of septic shock and cerebral malaria. Examples of TNF-α antibodies include anti-TNF and CB-006 and cA-2.

6. Soluble TNF-α receptors

Soluble TNF-α receptors bind both membrane-bound and soluble TNF-α. Clinical results with these agents in rheumatoid arthritis and allergic inflammation[59] are promising. Examples include soluble P^{75} TNF-α receptor and human IgG fusion protein (sTNF-αR.Fc), Poly Ethylene Glycol linked P^{55} (PEG-sTNF-R1) and RO-45-208 (Tenefuse).

APOPTOSIS

Apoptosis is a process of programmed cell death, or rather cell suicide, which is essential for the proper functioning of the body. The term apoptosis was used by Kerr & co-workers in 1972. In classical Greek apoptosis means "dropping off", as in the dropping off of flower petals or falling leaves[60].

Health of all multicellular organisms, including humans, depends not only on the body's ability to produce new cells also on the ability of individual cells to self-destruct when they become superfluous or disordered. It appears that aberrant regulation of apoptosis - leading to too much or too little cell suicide - probably contribute to various disorders such as cancer, AIDS, Alzheimer's disease and rheumatoid arthritis.

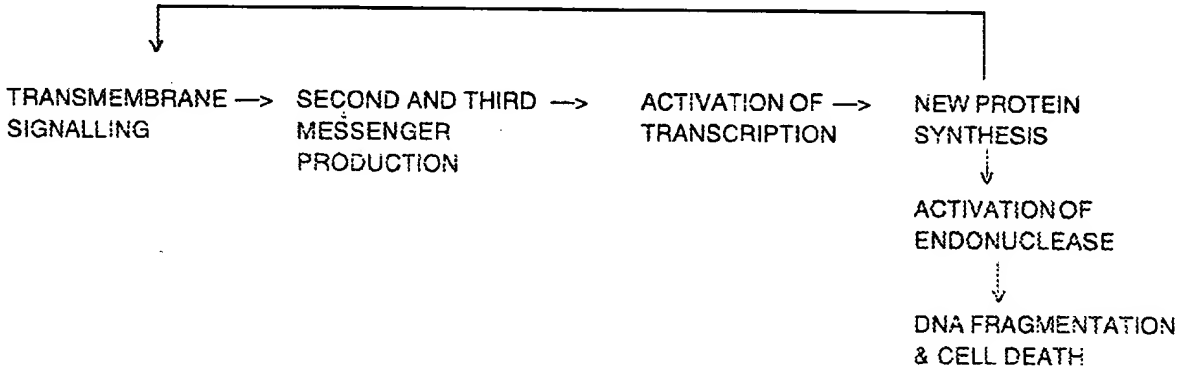
Apoptosis has been ascribed roles in embryogenesis, differentiation, ageing, metamorphosis, neural development, epithelial turn over of skin and gut lining tumor regression, etc. In our living system, apoptosis serves the following function-

1. Deletion of cells that are required only for a given time in tissue development and function
2. Elimination of redundant cells to keep cell number constant
3. Removal of transformed cells to prevent tumorigenesis.

Virtually all tissue harbor apoptotic cells at one time or another. The cells usually commit suicide for the greater good of the body. The list of examples is far from exhaustive. A few of these include the eye, where the lens consist of apoptotic cells that have replaced their innards with the clear protein crystalline; the thymus, where T lymphocytes mature and those that are auto reactive are deleted; the uterus, where the cells of the uterine wall sloughed off during menstruation perish by apoptosis; and other cells, where they undergo suicide when infected by virus or sustain irreparable genetic mutation.

Cell dying through apoptosis undergoes destructive morphological changes[61]. First it pulls and shrinks away from its neighbours, then blebs appear on the surface making the cell appear to boil. This phenomena is also known as zeiosis. The chromatin condenses at the edge of nucleus in the form of crescent. Soon the nucleus breaks up and gets enveloped by cell fragments to form the apoptotic bodies. The apoptotic bodies are quickly ingested by other cells in the vicinity or by macrophages. The hallmark of apoptosis is the internucleosomal cleavage resulting in oligonucleosomal DNA ladder fragments of approximately 185 base pair[62]. Initially the process of programmed cell death was called 'shrinkage necrosis'. In contrast with necrosis, several investigators have identified numerous features that distinguishes apoptosis (Table 5)[63-65]. The molecular mechanisms during apoptosis is ill-defined and there is no uniform sequence of metabolic events that occur during apoptosis. It is possible at the present stage to integrate all the available data into a single acceptable concept.

On the whole the events during apoptosis could be summarised as -



Apoptosis and Inflammation

Following tissue injury, inflammatory cells invade the lesion; then fibroblast migrate, proliferate, and synthesize extracellular matrix component participating in the formation of granulation tissue. As the wound closes and evolves into a scar, there is an important decrease in cellularity and in particular myofibroblast disappear[66]. This cellular loss is brought about by apoptosis. In chronic inflammatory conditions like rheumatoid arthritis T-cells migrate into the synovium where they fail to die, resulting in increased T-cell number. Synovial cells in rheumatoid patients undergo extensive DNA fragmentation. However, these cells fail to complete apoptosis thus permitting chronic proliferative arthritis to continue unabated[67].

Fig. 4 shows how induction of apoptosis may be beneficial in the treatment of chronic inflammatory conditions. An inflammatory response is usually amplified by neutrophils, a cell loaded with potent proteolytic enzymes, toxic cationic proteins and chemotactic factors. Rather than being programmed to die by necrosis, which allows uncontrolled escape of pro-inflammatory contents, if these cells could be removed by apoptosis, which effectively protects the surrounding tissue from neutrophil content, it would lead to cessation of inflammation.

Elimination by apoptosis of both infiltrating leukocytes and proliferating resident cell suggest an important mechanism for promoting resolution of inflammation and guarding against post-inflammatory scarring[68]. Deletion of myofibroblasts, involved in the

Table 5: Comparison of Apoptosis and Necrosis

APOPTOSIS	NECROSIS
• functional form of cell death	• accidental form of cell death
• occurs under physiological condition	• seen under pathological condition
• active death requiring the cell to expend energy towards its own demise	• cell is passive during necrosis
• cell shrinks and pulls away from its neighbours	• cell swelling in a defining feature
• nucleus ruptures	• entire cell balloons and ruptures
• contents of dying cell remain sealed are with in vesicles until apoptotic bodies removed through phagocytosis.	• showering of neighbouring cells with cellular debris of necrosis
• no inflammation follows apoptosis	• necrosis is followed by inflammation.

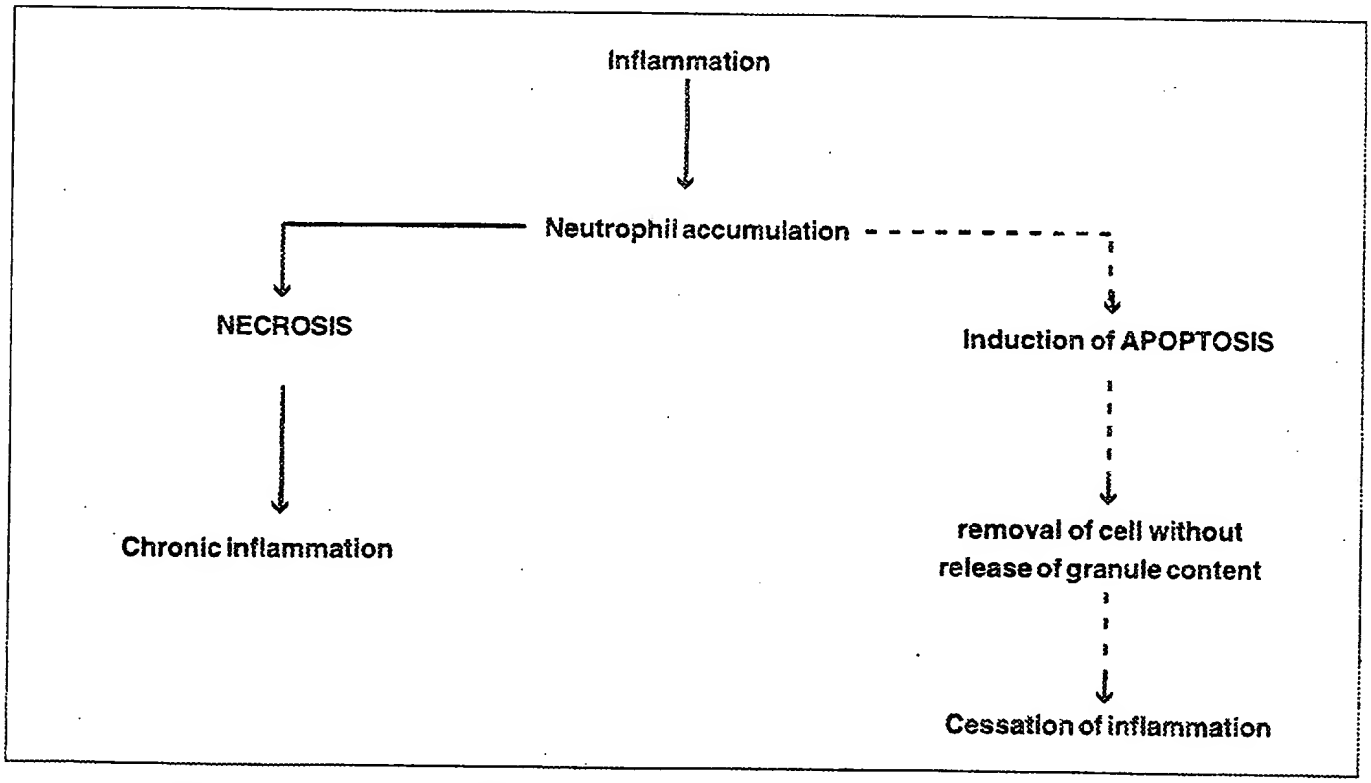


Fig. 4: Induction of apoptosis (---->) in proinflammatory cells could lead to cessation of inflammation while necrosis (—>) leads to chronic inflammation

repair of skin wounds, when healing ensues by apoptosis prevents formation of hypertrophic scars[69]. Excess glomerular mesangial cells resulting from proliferation due to glomerular injury can be cleared away by apoptosis so that glomerular structure and function return to normal[70]. Apoptosis can also be beneficially exploited to prevent fibrosis in patients with chronic bronchopulmonary dysplasia. Chronic airway inflammation in asthma can also be resolved through induction of apoptosis of Th2 (T helper 2) cells[71], which drives cycles of tissue damage mediated by degranulation products released from large number of eosinophils, via the release of cytokines.

Since apoptosis can be induced by several mechanism several attractive approaches could serve as potential therapeutic strategies. These include-

a) **Fas Mimetics :** Fas is a cell surface protein expressed on the T cells when it encounters an antigen. They also temporarily make another surface molecule called Fas ligand. In activated T cells Fas bind Fas ligand thereby signalling the cell to undergo apoptosis. Thus we could hypothesize that compounds that bind FAs can initiate apoptosis.

b) **Anti Fas/APO-1 antibody** : Monoclonal antibodies against Fas or APO-1 (apoptosis inducing protein - 1) react with cell surface molecule, APO-1, and trigger signals inducing apoptosis.

c) **Cytokine/growth factor blockers :** Drugs inhibiting the synthesis or receptor binding of cytokine/ growth factors can trigger passive apoptosis.

d) **Integrin inhibitors :** Integrins are primarily responsible for adhesion to extracellular matrices. Extra cellular matrix interaction blockers like integrin inhibitors deny anchorage and induce apoptosis[72].

e) **Poly(ADP) ribose polymerase activators :** Activation of Poly(ADP) ribose polymerase results in consumption of NAD in course of polymer formation leading to decline in ADP levels resulting in breakdown of glycolysis; ultimately leading to cell death.

f) **Lamin and Actin blockers :** Lamin and Actin are proteins involved in chromatin condensation and maintaining cell membrane architecture. Drugs which block these would, however, induce apoptosis indiscriminately.

g) **Topoisomerase inhibitors** : Topoisomerase inhibitor stabilizes enzyme-DNA complex leading to the production of single- or double- strand breaks that ultimately precipitate cell death.

h) **Nucleoside analogues** : Nucleoside analogues also induce DNA strand breaks, although their mechanism of action may additionally involve cellular signalling network. This has been shown by purine nucleoside analogues in chronic lymphocytic leukaemia (CLL) cells[73].

i) **Anti-apoptotic gene inhibitors :** Bcl-2, Bcl-X_L, Bcl-X_β, Bcl-W, bcr-abl, V-abl are examples of genes that inhibit apoptosis. Inhibition of expression of these genes would facilitate the induction of apoptosis. Antisense oligonucleotide would be the most suitable approach in this regard.

The discovery of new methods of selectivity inducing apoptosis may lead to entirely new therapies for chronic inflammatory diseases.

CONCLUSION

The development of selective COX-2 inhibitors seems promising and could be of potential use in clinical situations in which COX-2 is overexpressed. COX-2 overexpression is implicated in colorectal

cancer and neurodegenerative diseases. Thus selective inhibitors might have preventive and/or thereapeutic effects and those that pass through blood brain barrier could have therapeutic potency in stroke and Alzheimer's disease. COX-2 inhibitors have no greater efficacy as antiinflammatory agents than classical NSAIDs, but their safety is clearly much higher.

The inflammatory diseases where TNF-α appears to play a pathological role, the goal is to neutralise TNF-α or down regulate its production. Biotechnology has provided neutralizing antibodies to TNF-α and the use of recombinant soluble TNF receptor antagonist is being pioneered. It is hoped that near future will yield a number of safe and effective medicines useful in combating TNF-α and its pathological effects in a variety of inflammatory diseases.

Apoptosis has provided an insight into how chronic inflammatory conditions can be mitigated by circumventing the process of necrosis. These are still early days in the study of cell suicide, and so efforts aimed at treating disease by manipulating the process are also at relatively early stage. The growing understanding of apoptosis should greatly enhance the efforts toward a better remedy for chronic inflammatory conditions.

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SCIENTISTS AND CLINICIANS GET TO THE HEART OF THE MATTER

Facilitating the translation of findings made by molecular and cellular biologists into clinical practice was one of the aims of the European Science Foundation Workshop on Cardiovascular Specific Gene Expression held in Maastricht, the Netherlands.

The cloning of new cardiovascular genes and their regulatory sequences is usually driven by a desire to examine basic biological questions. The heart can be thought of as a series of modular transcriptional domains, explained Robert Kelly (Paris, France). This has provided the stimulus to identify region-specific genes. Identification of e-HAND, d-HAND, and CARP has, in turn, yielded markers for tracking processes of development and disease.

The description of gene expression patterns gives some insight into gene function, but a more complete understanding requires direct manipulation of gene expression or function. Consequently, gene targeting in the cardiovascular system was a major theme of the meeting. Peter Carmeliet (Leuven, Belgium) explained how planned gene modifications in mice can be used to dissect complex signalling networks and to analyse interactions between circulating mediators of thrombosis and the vascular wall. But fascinating cardiovascular phenotypes can also arise unexpectedly in such experiments. The muscle LIM transcription factor was first identified in skeletal muscle, said Pico Caroni (Basel, Switzerland) but deletion of the gene produced a mouse with the features of human dilated cardiomyopathy. This phenotype, like many others produced in gene-targeting experiments, is dependent on the mouse line in which the deletion is expressed. Identification of the host alleles determining susceptibility to the phenotype is now a major focus of research.

The heart and vasculature offer unique opportunities to examine in vivo how gene expression relates to function. The logic of pursuing quantitative physiological measurements in genetically engineered mice is widely appreciated, commented Hein Wellens (Maastricht, the Netherlands). Nevertheless, the full potential offered by these systems can only be realised with better communication between basic scientists and clinical cardiologists, he added.

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